Journal of Aquaculture 10 (4) : 403~407 (1997)

Genetic Stock Identification of Common Carp (*Cyprinus carpio*) by Detection of Intraspecific DNA Sequence Variation in the Mitochondrial 12S rRNA Gene

Yoon Kwon Nam, Su Dong Chu*, Chang Hwa Jeong, Choong Hwan Noh**, Jae-Yoon Jo and Dong Soo Kim

Department of Aquaculture, Pukyong National University Pusan 608-737, Korea
**Ministry of Eduction, Seoul 110-760, Korea*
***Living Resources Lab., KORDI, Seoul 425-600, Korea*

미토콘드리아 12S rRNA 유전자 변이 조사를 통한 잉어(*Cyprinus carpio*)의 유전학적 동정

남윤권 · 주수동* · 정창화 · 노충환** · 조재윤 · 김동수

부경대학교 양식학과, *교육부
**한국해양연구소 생물자원개발연구실

Intraspecific sequence variation was detected by polymerase chain reaction (PCR) and direct sequencing of a 350-nucleotide region of the mitochondrial 12S rRNA gene of two natural populations (Han River and Nakdong River) and one hatchery stock (Jinhae Inland Fisheries Institute) of local strain common carp, one Israeli strain of common carp stock from Pukyong National University (PKU), and one hybrid between Israeli strain of common carp female and local strain common carp male from PKU stock. There is little variation in 350 bases of the mitochondrial 12S rRNA gene sequences among 2 natural and 1 hatchery local strain common carp populations, representing about 7 to 20 nucleotide differences (less than 6%). The sequence of specimens from Han River was more similar to that from Nakdong River (identity=98.0%) than to that from Jinhae Inland Fisheries Institute (identity=96.3%). Sequence variation between Israeli strain and wild local strain common carp was higher than the variation within natural stocks. The level of variation was ranged from 15.7 to 17.7%. The hybrid showed very similar nucleotide sequence of 12S rRNA gene to the sequence of Israeli strain with the identity of 98.9%.

Key words : Common carp, PCR, Direct sequencing, Intraspecific variation, mt12S rRNA gene

Introduction

The ability to identify genetically discrete stock of fish is imperative for effective fishery management (Funkenstein et al., 1990). The genetic delineation of such stock could solve

This study was supported by grants from Fisheries and Ocean Research Program, Ministry of Education.

current major problems of carp in Korea, which include a general decline in the productivity of cultured carp during the last decade. The major reason for decreased productivity of carp culture is being thought to be due to the accumulation of recessive trait, and the indiscreet cross breeding.

To investigate the genetic basis of stock or population structure of the exploited fish species, the genetic characters demonstrating sufficient variation to permit discrimination of intraspecific groups or stocks should be studied (Hynes et al., 1989). The discrimination of intraspecific groups or stocks has come from comparative studies of morphological characters. However, it is well documented that such characters are environmentally labile. Protein electrophoresis has a limited ability to resolve only a fraction of existing amino acid substitutions and detect the variations in only the part of genome coding for soluble enzymes. Restriction analysis of mitochondrial DNA has been widely used in order to study variation in other parts of the genome including non structural genes for elucidating the genetic relatedness existing within and between populations of many fish species. This technique has already provided much useful information on population structure and hybridization events (Hynes et al., 1989).

However, the maximum information can be obtained by looking directly at the order of nucleotides in the DNA chain (McVeigh et al., 1991). The unique properties of nucleotide sequence polymorphism of mitochondrial DNA (mtDNA) can provide the most useful information to understand the genetic structures of populations as well as glimpses into the recent evolutionary history of fish population, which may be helpful for the efficient management of breeding program (McVeigh et al., 1991 ; Ovenden et al., 1993). A recent advance in biotechnology, the polymerase chain reaction (PCR) and automated sequencing has also made it possible to examine the sequence polymorphism of a DNA sections routinely (Kocher et al., 1989).

The objective of this study is to provide the genetic basis information of several local and Israeli common carp stocks for genetic stock identification and selective breeding programs. We surveyed intraspecific DNA sequence variation in the mitochondrial 12S rRNA gene of the carp populations by polymerase chain reaction/direct sequence analysis.

Materials and methods

Sample collection

Fish specimens of local strain common carp (*Cyprinus carpio*) were sampled from Han River and Nakdong River as wild stocks and from Jinhae Inland Fisheries Institute as a cultured stock. Fish samples of Israeli strain of common carp and intraspecific hybrid between Israeli strain (♀) and local strain common carp (♂) also collected from Culture Station of Pukyong National University. About 20 individuals from each of the populations were collected and analysed.

DNA extraction

Total cellular DNA were isolated from either of 2 ul of whole blood, 1 mm of babel or a scale. The tissue sample was digested by conventional SDS/proteinase K method. Briefly, DNA sample in a solution of 50 mM Tris, 5 mM ethylendiaminetetracyclicacetic acid, sodium salt (EDTA-Na), pH 8.0, 150 mM NaCl, 0.5% sodium dodecyl sulfate (SDS), 200 μg/㎖ proteinase K were incubated at 50℃ for several hours to overnight until the sample was digested. DNA samples were extracted once with TE-saturated phenol (pH 8.0), and twice with phenol/chloroform/isoamyl alcohol (25 : 24 : 1), and then precipitated with equal volume of isopropanol. DNA pellet was washed once with 70% ethanol and dissolved in 1x TE (10 mM Tris, 1 mM EDTA-Na, pH 8.0).

Amplification of mitochondrial 12S rRNA fragment by PCR

Approximately 0.2 μg of boiled genomic DNA was used in a PCR reaction. PCR reaction mixture contained 20 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 25 mM KCl, 100 μg/㎖ gela-

tin, 20 pmoles of each PCR primer, 50 μM of each dNTPs, and 2.5 U Taq DNA polymerase (Perkin Elmer Co.). The temperature sequence for amplifying the 12S rRNA gene was according to Whitmore et al. (1992) with slight modification. The reaction was carried out at the following procedure ; 94℃ for 1 min., 50℃ for 1 min, 72℃ for 1 min. for 30 cycles with initial 94℃ denaturation step for 1 min. Two oligonucleotide primers (L 1091 and H1478) were designed to amplify the fragments of 12S rRNA gene as described by Whitmore et al. (1992).

Sequence analysis

Sequencing reaction were performed on purified PCR products automatic sequencer (Perkin Elmer Co., USA) for direct determination of sequences of amplified 12S rRNA segments. The detail procedures were according to the manufacturer's recommendations. A readable sequence of approximately 350 bases of the mitochondrial 12S rRNA gene was analyzed.

Results

Sequence variations among natural and cultured stocks of local strain common carp

The sequences of 350 bp for the 12S rRNA gene in the 3 local strain common carp populations are shown in Fig. 1. No notable sequence variation within a population was observed. There are 7 to 20 nucleotide differences among the 12S rRNA genes from the populations (Han River, Nakdong River and Jinhae Inland Fisheries Institute), and there are within the range of 2% (minimum) to 5.5% (maximum). Substitution of nucleotide by transition was dominant in all 3 populations. The 12S rRNA sequence of specimens from Han River was more similar to that from Nakdong River (identity=98%) than to that from cultured stock from Jinhae Inland Fisheries Institue

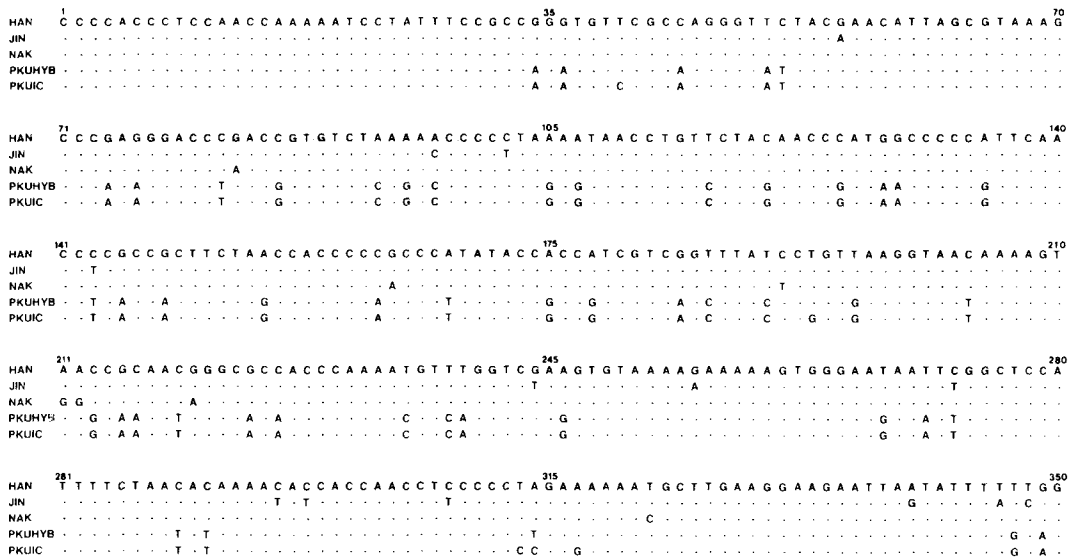

Fig. 1. Sequence alignments of mitochondrial 12S rRNA segments from several intraspecific populations of common carp. Bases are adenine (A), cytosine (C), guanine (G), and thymine (T). Abbreviations are : HAN, natural local strain common carp from Han River ; JIN, cultured stock of local strain common carp from Jinhae Inland Fisheries Institute ; NAK, natural common carp from Nakdong River ; PKUHYB, hybrid bewteen Israeli strain of common carp ♀ x common carp ♂ maintained in Pukyong National University ; PKUIC, Israeli strain of common carp maintained in Pukyong National Univerisity.

Table 1. Sequence homology (%) of amplified mitochondrial 12S rRNA gene segments from several intraspecific populations of common

	HAN	NAK	JIN	PKUHYB	PKUIC
HAN	100	98.0	96.3	85.4	84.3
NAK	–	100	94.3	83.4	82.3
JIN	–	–	100	83.4	82.3
PKUHYB	–	–	–	100	98.9
PKUIC	–	–	–	–	100

Abbreviations used are same as those described in Fig. 1.

(identity=96%) (Table 1).

Sequences of Israeli strain and hybrid

An alignment of 350 nucleotide sequence for 12S rRNA gene from Israeli strain of common carp and hybrid between Israeli strain (♀) and local strain common carp (♂) is also presented in Fig. 1. Nucleotide sequence from Israeli strain showed more divergent variation when compared to the variations among common carp population. Out of 350 bases analysed in the Israeli strain common carp, 55 bp to 62 bp (15.7~17.7%) were different to the sequence from the local strain common carp populations. The ratio of transition to transversion was closed to 3 : 2. Nucleotide sequence of the hybrid was very similar to that of Israeli strain with the identity of 98.9% (Table 1).

Discussion

One of the most common problems in fishery management is how to identify genetically meaningful management unit. Many recent approaches based on the genetic studies have provided a new dimension in attempts to obtain solution of the problems associated with the breeding management and selection programs of cultured species (Funkenstein et al., 1990 ; King et al., 1993).

Mitochondrial DNA of fish has been given much attraction as an useful genetic marker for studying the population structure and evolutionary survey because it is non-recombining and it can also provide a different perspective of population structure and dynamics compared to the nuclear genome. Mitochondrial genome has been most extensively examined by restriction fragment length polymorphism (RFLP) analysis of the entire mitochondrial genome, in which relatively large amount samples and invasive laborious procedure are common limitation. However, recent developments in molecular biology have made it quite feasible to determine the sequence of a gene from a large number of samples by means of PCR and direct sequencing (McVeigh et al., 1991 ; McVeigh and Davidson, 1991).

In this study, there are little variation in the mitochondrial 12S rRNA gene sequences among the 3 local strains of common carp populations. This result shows a close genetic relationship among them. The level of sequence difference is only less than 6%, and the majority of nucleotide differences are transition (A↔G or C↔T). This transition has been well known in the genetically closely related species and/or populations (Kocher et al., 1989). This transition bias may indicate that these populations have diverged from a common ancestor (Brown et al., 1982 ; Bartlett and Davidson, 1991).

However, relatively large amount of intraspecific variation in 12S rRNA gene between the local strain and Israeli strain was observed in this study. One possible, but undefined, explanation for this high level of intraspecific variation between them is that there had been large amount of diversity between the former populations from which they were derived, and successive re-stocking of Israeli strain in a limited number of hatcheries might be responsible for separation between wild population of common carp and imported hatchery stock of Israeli strain.

The hybrid fish between Israeli strain common carp (♀) and local strain common carp (♂) in PKU stock revealed the highest identity in the 12S rRNA genes, which representing an important feature of mitochondrial inheritance, the maternal transmission.

It may provide a unique view of the vector direction of introgression resulting from hybridization. However, further study should be needed to elucidate the genetic variation between domesticated local strain common carp stocks and Israeli strains in each hatchery because the possible hybridization events could occur probably. Future analyses, using extensive collection of samples including wild and cultured stocks are also required to clarify for both the more detailed geographic patterns of mtDNA variants and genetic assemblage and relationships between the populations of local and Israeli strain common carps.

References

Bartlett, S. E. and W. S. Davidson, 1991. Identification of tuna species in the genus *Thunnus* by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. Can. J. Fish. Aquat. Sci., 48 : 309-317.

Brown, W. M., E. M. Prager, A. Wang and A. C. Wilson, 1982. Mitochondrial DNA sequences of primates : tempo and mode of evolution. J. Mol. Evol., 18 : 225-239.

Funkenstein, B., B. Cavari, T. Stadie and E. Davisovitch, 1990. Restriction site polymorphism of mitochondrial DNA of the gilthead sea bream (*Sparus aurata*) broodstock in Eilat, Israel. Aquaculture, 89 : 217-223.

Hynes, R. A., E. J. Duke and P. Joyce, 1989. Mitochondrial DNA as a genetic marker for brown trout, *Salmo trutta* L., populations. J. Fish Biol., 35 : 687-701.

King, D. P. F., S. J. Hovey, D. Thompson and A. Scott, 1993. Mitochondrial DNA variation in Atlantic salmon, *Salmo salar* L., populations. J. Fish Biol., 42 : 25-33.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca and A. C. Wilson, 1989. Dynamics of mitochondrial DNA evolution in animals : amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA, 86 : 6196-6200.

McVeigh, H. P., S. E. Bartlett and W. S. Davidson, 1991. Polymerase chain reaction/direct sequence analysis of the cytochrome *b* gene in *Salmo salar*. Aquaculture, 95 : 225-233.

McVeigh, H. P. and W. S. Davidson, 1991. A salmonid phylogeny inferred from mitochondrial cytochrome *b* gene sequences. J. Fish Biol., 39 : 277-282.

Ovenden, J. R., R. Bywater and R. W. G. White, 1993. Mitochondrial DNA nucleotide sequence variation in Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) from Tasmania, Australia. Aquaculture, 114 : 217-227.

Whitmore, D. H., T. H. Thai and C. M. Craft, 1992. Gene amplification permits minimally invasive analysis of fish mitochondrial DNA. Trans. Am. Fish. Soc., 121 : 170-177.